# Infrared Chemical Imaging for Pathology and Forensic Biology

Fiona Burger

A thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy (Science)

**2013**

**Department of Chemistry and Forensic Science**

**University of Technology, Sydney**

# CERTIFICATE OF AUTHORSHIP / ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Fiona Burger

2013

## ACKNOWLEDGEMENTS

As with any large undertaking during one's life, the final product represents the work and dedication of not just the author, but the essential body of souls that each contribute, either directly or indirectly, to the research process as a whole.

Firstly to my supervisors and project partners thanks to whom which none of this work would ever have been possible without their valued time and input. To Dr. Brian Reedy, my primary supervisor over these many long years, thank you for sharing with me with your guidance and extensive pool of knowledge. Without your patience and perseverance with my erratic attendances I would still be stumbling in the dark. Thank you also to Dr. Philip Doble, my co-supervisor. To Dr. Richard Scolyer and Professor John Thompson of the Royal Prince Alfred Hospital Melanoma Research Unit, Mr. Kenneth Lai (formerly with the RPA Melanoma Research Unit), Dr. Neil Langlois (formerly with the Institute of Clinical and Medical Pathology Research - Westmead), Dr. Tamara Stzynda and Dr. Sarah Tarran, thank you for allowing me the time, samples and nevermind the whole impetus for these studies, as well as for always providing me with answers to my seemingly never-ending naïve pathology questions.

I must also mention the invaluable help that both Helen Rutlidge and Dominic Hare, my fellow postgraduate students at UTS, provided to me during the course of my research work. Helen, your ability to make the often daunting and incomprehensible nature of chemometric work achievable and logical to me may only be described as a phenomenal skill. Your ability to understand my data handling requirements and adapt numerous programs and statistical methods to my requirements was invaluable, as was your patience and friendliness throughout. Thank you also Dominic; not only for the assistance with my sample gathering and preparation, but for also giving me that gentle push (whether you intended it or not) to get my work moving along.

And finally to my family and friends I must deliver a huge thanks. To Christie Wallace-Kunkel and Garry Sarkissian, my fellow PhD peers; you have shown to me that the theory of a completing a PhD part-time is possible, no matter the work or personal interruptions that may fall in our path and that with perseverance anything is achievable. To Paul, my long-suffering partner, thank you for never letting me give up and always reminding me of the greater goals in life. Thank you to my parents who, like Paul, have had to put up with the good, the bad, and the ugly from me for so many years now, and who never failed to offer encouragement, love and support when I needed it the most. Also, a special thanks to my mother for allowing me to join her on her own melanoma journey.

# PREFACE

Tissue pathology assessment is primarily performed using simple human visual examination and interpretation. While various physico-chemical or biochemical treatments may be used to enhance or highlight components within the sample, the ultimate conclusion regarding the 'identity' of the sample lies with the very human examiner making comparisons with samples of known origin. Although this process sounds straightforward, more often than not the samples are less than ideal, and will be subject to natural variations which can introduce a whole gamete of artefacts which the examiner must integrate into their interpretation process. Suddenly, the comparison process goes from distinct, 'black and white' decisions, to an entire greyscale in-between.

Pathologists garner their knowledge not just from theory, but by training themselves over many years to recognise the normal from the abnormal. The pathologist must be able to deliver, with confidence, a diagnosis or ruling to a patient, treating medical professional, or (in some cases) a court. Their findings are based on a comparison of the subject tissue with many tissue samples of known prognosis, and the recognition of cell and tissue structures that 'do not belong'. Hence, it is years of exposure to endless numbers of cases which proves to be critical in achieving consistent and correct diagnoses.

Any tool that can improve the visual appearance of a sample in such comparison work is always of interest to the practitioner. Reducing the subjective nature of any scientific examination process, and more importantly reducing the potential for human error, is something all researchers strive for. The ability to confirm a pathological finding with an objective, computerised result, may also offer greater confidence to the information receiver, be they a patient or jury. It is the development of a more objective approach to tissue analysis that forms the fundamental premise of this work.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

## ABBREVIATIONS

| | |
|---|---|
| %CC | Percentage of Correctly Classified |
| AAS | Atomic Absorption Spectroscopy |
| AIHW | Australian Institute of Health and Welfare |
| AJCC | American Joint Committee on Cancer |
| ANN | Artificial Neural Networks |
| ALM | Acral Lentiginous Melanoma |
| ANOVA | Analysis of Variance |
| ASCII | American Standard Code for Information Interchange |
| ASD | Average-Standard Deviation |
| AUC | Area Under the Curve |
| Avg | Average |
| BA | Boric Acid |
| BCC | Basal Cell Carcinoma |
| BMU | Best-Matching Unit |
| BTEM | Band-Target Entropy Minimisation |
| CC% | Correct Classification Percentage |
| CCD | Charge Coupled Device |
| CDK | Cyclin/cyclin-dependent kinase |
| CI | Chemical Imaging |
| CLND | Completion Regional Lymph Node Dissection |
| CT | Computed Tomography |
| DNA | Deoxyribonucleic acid |
| ED | Euclidean Distance |
| EFOV | Expanded Field Of View |
| EGF | Epidermal Growth Factor |

| | |
|---|---|
| ELISA | Enzyme-linked Immunosorbent Assay |
| ELND | Elective Lymph Node Dissection |
| EMSC | Extended Multiplicative Signal Correction |
| FGF | Fibroblast Growth Factor |
| FIR | Far-infrared |
| FISH | Fluorescence *in situ* Hybridisation |
| FNB | Fine Needle aspiration Biopsy |
| FPA | Focal Plane Array |
| FRCPA | Fellow of the Royal College of Pathologists of Australasia |
| FTIR | Fourier Transform Infrared |
| FTIR CI | Fourier Transform Infrared Chemical Imaging |
| G0 | Gap 0 |
| G1 | Gap 1 |
| G2 | Gap 2 |
| GM-CSF | Granulocyte-Macrophage Colony-Stimulating Factor |
| H&E | Haematoxylin and Eosin |
| HCA | Hierarchical Cluster Analysis |
| HLA | Human Leukocyte Antigen |
| HPLC | High Performance Liquid Chromatography |
| ICP-MS | Inductively Coupled Plasma-Mass Spectrometry |
| IFN | Interferon |
| IGF | Insulin-like Growth Factor |
| IL | Interleukin |
| IP | Intraperitoneal |
| ISEL | *In Situ* End labelling |
| LA-ICP-MS | Laser Ablation Inductively Coupled Plasma Mass Spectroscopy |
| LDA | Linear Discriminant Analysis |

| | |
|---|---|
| LDH | Lactate Dehydrogenase |
| LN | Lymph Node |
| LOO | Leave One Out |
| LT | Leukotriene |
| LX | Lipoxin |
| MALDI-IMS | Matrix-assisted Laser Desorption Ionisation Imaging Mass Spectrometry |
| MCT | Mercury Cadmium Telluride |
| Med | Median |
| mf | Myofibroblasts |
| MF | Matched Filtering |
| Min/Max | Minimum / Maximum |
| MIQR | Median-Interquartile Range |
| MIR | Mid-infrared |
| MMP | Matrix Metalloproteinase |
| MNF | Minimum Noise Fraction |
| MQE | Mean Quantisation Error |
| MRDF | Maximal Multinomial Logistic Regression |
| mRNA | Messenger Ribonucleic Acid |
| MTMF | Mixture-Tuned Matched Filtering |
| MVN | Multivariate Normal |
| m/z | Mass to Charge Ratio |
| NADH | Nicotinamide Adenine Dinucleotide |
| NIR | Near-infrared |
| NMSC | Non-melanoma Skin Cancer |
| NN | Nearest Neighbour |
| NOR | Nucleolar Organiser Region |
| NR-Laser-SNMS | Non-resonant Laser Secondary Neutral Mass Spectrometry |

| | |
|---|---|
| NSW | New South Wales |
| OPD | Optical Path Difference |
| PAP | Papanicolaou |
| PAS | Periodic Acid-Schiff |
| PBS | Phosphate-buffered Saline |
| PC | Principal Component |
| PCA | Principal Components Analysis |
| PCR | Polymerase Chain Reaction |
| PDGF | Platelet-derived Growth Factor |
| PG | Prostaglandin |
| PPI | Pixel Purity Index |
| pTNM | Primary Tumour Node Metastasis |
| QDA | Quadratic Discriminant Analysis |
| RCPA | Royal College of Pathologists of Australasia |
| RGP | Radial Growth Phase |
| RNA | Ribonucleic Acid |
| ROI | Region Of Interest |
| RPAH | Royal Prince Alfred Hospital |
| RT-PCR | Reverse Transcriptase-polymerase Chain Reaction |
| SAM | Spectral Angle Measure |
| SCC | Squamous Cell Carcinoma |
| SCS | Spectral Correlation Similarity |
| SIMCA | Soft Independent Modelling of Class Analogy |
| SLNB | Sentinel Lymph Node Biopsy |
| SMLR | Sparse Multinomial Logistic Regression |
| SN | Sentinel Node (*aka* Sentinel Lymph Node) |
| SOM | Self Organising Map |

| | |
|---|---|
| SPF | Specific Pathogen Free |
| SSV | Spectral Similarity Value |
| SVM | Support Vector Machine |
| SVP | Spectral Vector Properties |
| TE | Topographic Error |
| TGF | Transforming Growth Factor |
| THSIS | Thermal and Hyperspectral Imaging System |
| TIL | Tumour Infiltrating Lymphocyte |
| TNF | Tumour Necrosis Factor |
| TNM | Tumour (**T**) / Lymph Nodes (**N**) / Metastasis (**M**) |
| TOF-SIMS | Time-of-flight Secondary Ion Mass Spectrometry |
| UTS | University of Technology, Sydney |
| UV | Ultraviolet |
| UV-Vis | Ultraviolet-Visible |
| VCA | Vertex Component Analysis |
| VGP | Vertical-Growth Phase |
| WLE | Wide Local Excision |

# ABSTRACT

The objective of this research was to explore the capability of Fourier Transform Infrared Chemical Imaging (FTIR CI) for two specific pathology applications: 1) the analysis of human tissues for the diagnosis of melanoma, and 2) incised skin wound age determination for the purpose of forensic investigation.

For the melanoma study, thin serial sections were obtained from an archival tissue bank that consisted of pathologist pre-diagnosed ("gold-standard"), paraffin-embedded, human skin and lymph node tissues. Thin sections from each block were mounted on infrared reflective microscope slides and imaged, and a selection of the total images nominated as either training or test samples. Each training sample image was then compared to its corresponding haematoxylin and eosin (H&E)-stained section and reference library spectra extracted. Vertex component analysis (VCA) as a spectral feature extraction method was also explored. Classification of the test sample images was then performed using the spectral angle mapper (SAM) algorithm and the accuracy assessed by comparing the resulting classification images to the H&E-stained tissue sections. The tissue classification model developed produced a range in result quality, and highlighted various critical aspects in the construction of such methodologies. The taking of spectral derivatives improved image classification, as did the removal of paraffin from the tissue (although no data treatment targeting the paraffin was conducted on the non-deparaffinised tissues). Although the accuracy achieved in this study fell short of that required for clinical practice, the results obtained demonstrate that further investigation into the SAM algorithm as a tissue classifying tool is certainly warranted.

The second pathology application explored the ageing of wounds, a determination that may be critical in criminal investigations, particularly in homicide investigations, in which the timing of wound infliction may be crucial evidence. For this study, incised wounds were

inflicted on rats in a controlled manner and the tissue excised following a known amount of healing time ranging from 5 minutes to 288 hours (12 days). Thin sections of the wounds were mounted on infrared reflective slides, deparaffinised and then imaged using FTIR CI. Although four classification models were attempted, none were capable of producing highly accurate wound age determination. Spectral variation was observed between earlier and later wound ages using some of the classification methods, but the ability to correctly group the test samples into their respective age groups was not achieved. Based on the number of variables which must be taken into consideration when performing such a study, and the number of areas identified as needing further improvement (e.g. spectral data quality), the fact that even a limited form of discrimination was achieved using FTIR CI was encouraging.